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Donor Lymphocyte Infusion May Reduce the Incidence of Bronchiolitis Obliterans after Allogeneic Stem Cell Transplantation

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Bronchiolitis obliterans (BO) is a serious pulmonary complication after allogeneic hematopoietic stem cell transplantation (HSCT). The aim of this study was to evaluate the diagnostic methods used, the incidence of BO, risk factors, and outcome in patients with BO at our center. The study included 527 HSCT patients transplanted between 1995 and 2003. Lung function tests (n = 1177) and risk factor analyses were performed in all patients. Chest X-rays and high-resolution tomographies were investigated in patients with BO. The incidence of BO was 4.8%, as the diagnosis was established in 25 patients (4 children). Median time between HSCT and diagnosis of BO was 356 (84-1823) days. Eight patients (32%) had radiologic changes consistent with BO. Forced expiratory volume for 1 second (FEV_1) and forced expiratory flow at 50% (FEF_{50}) and 75% (FEF_{75}) of forced vital capacity (FVC) produced median values that were 49%, 25%, and 18% of the reference values at the time of BO diagnosis. FEF_{75} was reduced before BO diagnosis in 7 patients (28%). In a multivariate risk factor analysis, chronic graft-versus-host disease (cGVHD) was found to be associated with BO ($P < .001$), whereas donor lymphocyte infusion (DLI) diminished the risk ($P = .02$). For 10 patients with late BO (>1 year after HSCT), 80% survived 5 years after diagnosis, compared to 38% survival in 15 patients with early-onset BO ($P = .06$). We conclude that lung function tests with a persistent decrease in FEV_1 were more important than radiographic methods to recognize and monitor BO, that FEF_{75} may serve as an early warning of BO, and that late-onset BO appears to be associated with better outcome. Chronic GVHD was confirmed as a risk factor, and administration of DLI may diminish the risk.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has become a widely accepted treatment for malignancies and lymphohematopoietic failure syndromes. Despite the advances in histocompatibility matching, new and better treatments for infectious complications, and better immunosuppressive drug treatments, pulmonary complications have continued to be common and often lethal [1-4]. Bronchiolitis obliterans (BO) is a serious, noninfectious pulmonary disorder associated with a poor prognosis [1-3,5]. The underlying pathogenic mechanisms are poorly understood, and the etiology remains obscure (1,3). The most important factor associated with BO has been shown to be the presence of chronic graft-versus-host disease (cGVHD) [6-9]. BO is histologically characterized by obliteration of the lumen of the respiratory bronchioles by organizing granulation tissue, infiltration of mononuclear cells, or fibrosis, and clinically by persistent, progressive obstruction of air flow [1,3,5,10]. In this retrospective single-center study, we determined the incidence of BO, risk factors, and outcome in HSCT recipients who developed this complication, and we evaluated the use of radiology and pulmonary function tests to establish the diagnosis.

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PATIENTS AND METHODS

Patients and Donors

Between January 1995 and December 2003, 527 patients underwent allogeneic HSCT at Karolinska University Hospital, Huddinge. Seventy-seven patients died within 120 days of HSCT, and 102 patients

had insufficient follow-up data because they lived in other countries or because of the lack of lung function tests. In the remaining 330 patients, a total of 1177 lung function tests were performed. For these 330 patients, all spirometries were carefully studied and re-evaluated by an experienced physiologist and a lung specialist. Patients with spirometries showing no or minimal air flow obstruction with normal or nearly normal forced expiratory volume for 1 second (FEV_1), forced expiratory flow at 50% (FEF_{50}), and 75% (FEF_{75}) values were regarded as non-BO cases. Patients with mild, moderate, or severe obstruction of air flow (FEV_1 66%-80%, 51%-65%, or ≤50%) [1,11] were investigated concerning clinical findings and radiographic, pathologic, and laboratory data. Cases that did not fulfill the National Institutes of Health (NIH) criteria for BO [12] were then screened as non-BO cases.

There were 295 male patients and 232 female patients, with a median age of 34 years (range: <1 to 77). Most patients had malignancies (n = 471), mainly acute leukemia (n = 242) or chronic leukemia (n = 110). Donors were an HLA-identical sibling or related individual (n = 227), an HLA-A, -B, and -DRB1 matched unrelated donor (MUD) (n = 246), or a mismatched related/unrelated donor (n = 54). Nonmalignant disorders included severe aplastic anemia (SAA) (n = 23), Fanconi's anemia (FA) (n = 5), paroxysmal nocturnal hemoglobinuria (PNH) (n = 2), and various inherited metabolic disorders. Polymerase chain reaction (PCR) sequence-specific primer (SSP) high-resolution typing was used for both HLA class I and II antigens [13].

The study was approved by the ethics committee of Karolinska Institutet (DNR 425/97). Characteristics of donors and of patients with or without BO are given in Table 1.

Conditioning and GVHD Prophylaxis

Myeloablative conditioning was administered to 417 patients and consisted of cyclophosphamide (Cy) at 120 mg/kg in combination with 7.5-10 Gy single-dose total-body irradiation (TBI) (n = 172) or fractionated (fTBI) 4 × 3 Gy (n = 94) (TBI-based) or busulfan (Bu) at 16 mg/kg (n = 136). Ten patients with SAA received Cy at 200 mg/kg, and 5 with FA

Table 1. Characteristics of Patients Who Underwent Allogeneic Hematopoietic Stem Cell Transplantation in the Period 1995 to 2003, According to Whether or Not They Developed Bronchiolitis Obliterans (BO)

	All Patients	BO	No BO
Diagnosis	n = 527	n = 25	n = 502
Nonmalignant disorder	56	1 (4%)	55 (11%)
Acute leukemia	242	12 (48%)	230 (46%)
Chronic leukemia	110	8 (32%)	102 (20%)
Other malignancy	119	4 (16%)	115 (23%)
Disease stage (early/late)	259/229	15/10	244/219
Sex (M/F)	295/232	14/11	281/221
Age	34 (<1-77)	33 (6–64)	34 (<1-77)
Donor			
HLA-identical related	227	12 (48%)	215 (43%)
MUD	246	11 (44%)	235 (47%)
Mismatched	54	2 (8%)	52 (10%)
Donor sex (M/F)	305/220	12/13	293/207
Donor age	37 (0-71)	37 (19-66)	37 (0-71)
Stem cell source (BM/PBSCs)	280/247	10/15	270/232
NC dose ($\times 10^8$/kg)	4.6 (0.03-80)	8.5 (0.7-25.6)	4.6 (0.03-80)
Female donor to male recipient	101 (19%)	6 (24%)	95 (19%)
G-CSF after HSCT	341 (65%)	20 (80%)	321 (64%)
Previous HSCT (auto/allo)	42 (8%)	3 (12%)	39 (8%)
Conditioning			
MAC	417	23 (92%)	394 (78%)
TBI-based	266	13 (52%)	253 (50%)
Non-TBI	151	10 (40%)	141 (28%)
RIC	110	2 (8%)	108 (22%)
ATG	349 (66%)	14 (56%)	335 (67%)
GVHD prophylaxis			
CsA + MTX	430 (82%)	23 (92%)	407 (81%)
CsA + MMF	45	0	45
Other	52	2 (8%)	50
DLI treatment	126 (24%)	2 (8%)	124 (25%)*
Acute GVHD II-IV	155 (29%)	7 (28%)	148 (30%)
Chronic GVHD	194 (37%)	22 (88%)	172 (34%) **
Follow-up (years)	7.0 (3.2-12.1)	7.6 (3.2-11.0)	7.0 (3.2-12.1)

BM indicates bone marrow; PBSCs, peripheral blood stem cells; NC dose nucleated cell dose; G-CSF, granulocyte-colony stimulating factor; SCT, stem cell transplantation; MAC, myeloablative conditioning; TBI, total-body irradiation; RIC, reduced-intensity conditioning; ATG, antithymocyte globulin; GVHD, graft-versus-host disease; CsA, cyclosporine A; MTX, methotrexate; MMF, mycophenolate mofetil; DLI, donor lymphocyte infusion.
Disease stage: early, CR1/CP1 (first complete remission/first chronic phase), or nonmalignant disorder; late, beyond CR1/CP1; MUD, HLA-A-, -B-, and -DRB1 matched unrelated donor.
*$P = .05$, ** $P < .001$.

received fludarabine (FLU) and Cy. Reduced-intensity conditioning (RIC) was given to 110 patients and consisted of FLU at 30 mg/m^2/day for 3 to 6 days in combination with Cy at 60 mg/kg for 2 days (n = 19), Bu at 4 mg/kg/day for 2 days (n = 46), treosulfan at 12 to 14 g/m^2/day for 3 days (n = 4), 2 Gy TBI (n = 34), or 2 × 3 Gy fTBI and Cy at 30 mg/m^2 for 2 days (n = 7) [14]. Antithymocyte globulin (ATG) was given for 2 to 5 days before transplantation to all patients with unrelated or mismatched donors, or with a nonmalignant disorder (n = 349) [15]. As GVHD prophylaxis cyclosporine A (CsA), and methotrexate (MTX) were given to the majority of patients (n = 430, 82%). During the first month, blood CsA levels were kept at 100 ng/mL when a sibling donor was used, and at 200 to 300 ng/mL when an unrelated donor was used. In the absence of GVHD, CsA was discontinued after 6 months for patients with malignancies and after 24 months for patients with nonmalignant disorders.

Diagnosis and Treatment of GVHD

Acute GVHD (aGVHD) and cGVHD were diagnosed and graded on the basis of clinical symptoms and/or biopsies (skin, liver, gastrointestinal tract, or oral mucosa) according to standard criteria [16,17]. The patients were treated for grade I aGVHD with prednisolone, starting at 2 mg/kg/day, which was tapered after the initial response. Chronic GVHD was initially treated with CsA and steroids.

Additional Treatment

Donor lymphocyte infusions (DLIs) were administered to 126 patients (24%) because of relapse (n = 62), increasing chimerism (n = 41), graft failure (n = 16), posttransplant lymphoproliferative disorder (PTLD) (n = 2), or as prophylactic treatment in patients with high-risk leukemia (n = 5). A median of 2 (range: 1-11) doses was given. The escalations in doses were done in steps of 0.5 to 1 log, starting between 1 × 10^5 to 1 × 10^6 CD3$^+$ cells/kg, depending on the type of donor, the degree of HLA match, and the history of GVHD in the recipient. DLI doses were given at approximately 4-week intervals until response or GVHD. First DLI was given at a median of 167 days (range: 23-1973) after HSCT.

Granulocyte colony-stimulating factor (G-CSF) (5 μg/kg/day) was given to all patients from day 10 after HSCT until neutrophil engraftment (>0.5 × 10^9/L) between April 1997 and August 2001. After that date, prophylactic G-CSF was not given because we found that there was an increased risk of aGVHD of grades II-IV with this drug.

Definition of BO

In this study, we followed the clinically diagnostic criteria for BO according to the NIH consensus document [12]. We defined BO as a severe pulmonary complication affecting the small airways, characterized by the onset of new airflow obstruction following allogeneic HSCT. Patients who developed BO within 1 year of HSCT were defined as having an early BO, and those who developed BO after 1 year were defined as having a late BO. The diagnosis required identification of pulmonary symptoms not explained by an infection, such as dry cough and dyspnea combined with a persistent reduction in FEV_1 in pulmonary function tests by ≥20% from baseline or FEV_1 <75% of that predicted, FEV_1/FVC <0.7 and residual volume (RV) >120%, radiographic changes consistent with BO, or pathological confirmation of constrictive bronchiolitis [7,12,18].

Diagnostic Methods

Spirometries were performed before transplantation, then approximately every 3 to 6 months during the first 2 years, and then at least once a year. Where there were respiratory symptoms such as dry cough or dyspnoea not explained by an infection, additional spirometries were conducted. FEV_1 and FVC, FEV_1/FVC ratio, FEF at 50% of FVC (FEF_{50}), and FEF at 75% of FVC (FEF_{75}) were also registered, as well as RV, total lung capacity (TLC), and diffusing lung capacity (DLC). FEF_{50} and FEF_{75} values represent maximal expiratory flow measured at points when 50% or 75% of the FVC has been expired.

Before transplantation, the majority of the patients had chest X-rays. Posttransplantation chest X-rays and high-resolution computed tomography (HRCT) was only performed when there were respiratory symptoms. In BO patients, chest X-rays are often normal. HRCT may, however, show multilobular areas with diminished attenuation and perfusion and/or air trapping at full expiration. Bronchiectasies (dilated and thickened bronchi) and areas of scarring may also be found. Sometimes a mosaic pattern is seen because of reduced perfusion to areas with obstruction and redistribution of the blood to normal areas [19,20]. However, previous studies have shown that radiographic findings in BO patients following HSCT and lung transplantation may be inconsistent and of limited accuracy [19,21].

In a few cases, bronchoalveolar lavage (BAL) was performed as a consequence of GVHD engaging the lungs. Otherwise, BAL was performed if an infection was suspected in BO patients. Video-assisted thoracoscopic surgical lung biopsies or transbronchial biopsies (TBBs) were not obtained because BO is often a patchy peripheral disease, a condition that makes it difficult to obtain an adequate biopsy sample [5,11], and because of the risk of bleeding and pneumothorax in these fragile and often thrombocytopenic patients [22,23].

Statistical Methods

The incidences of BO were estimated with cumulative incidence curves, taking competing events into consideration and compared by Gray's test. The competing event was death without BO. Patients with incomplete follow-up were censored at the time of last contact. Univariate and multivariate risk factor analyses were performed using the proportional subdistribution hazard regression model developed by Fine and Gray. Factors with P values of ≤.10 in the univariate analyses were introduced into the stepwise-elimination multivariate analyses. To study the effects of chronic GVHD and DLI as time-dependent variables, multivariate analysis was also performed using Cox proportional hazards model including time-dependent variables. Analysis was performed using the cmprsk package (developed by Gray, June 2001), Splus 6.2 software (Insightful, Seattle, WA), and Statistica software (StatSoft, Tulsa, OK). The Mann-Whitney U test was used to compare continuous variables and the chi-square method was used to compare the distribution of categoric variables.

RESULTS

General Data on Patients with BO

The cumulative incidence of BO in the 527 patients was 4.8%: 25 patients developed BO between 1995 and 2003. Median age was 33 (6-64) years and 4 patients (16%) were children aged 6 to 13 years (Table 1). Seven patients (28%) were smokers or ex-smokers, and 14 (56%) had never smoked. Smoking data were unavailable for 4 patients. Median time from HSCT to development of BO was 356 (84-1823) days. Acute GVHD of grades I (n = 11) and grades II-III (n = 7) occurred in 18 (72%) of the patients who developed BO and cGVHD in 22 (88%). The characteristics of the BO patients are given in Table 1.

Evaluation of Radiology and Spirometry in BO Patients

HRCT (n = 50) was performed median 2 (1-7) times after HSCT in 22 of the 25 patients with BO. Chest X-rays alone were performed in 3 patients. However, in these patients, RV was 159%, 175%, and 280%, respectively, which is why all of these 25 patients fulfilled the NIH criteria for BO despite the fact that no HRCT was performed. In 8 of 25 patients (32%), radiographic changes associated with BO were seen: bronchiectasies with dilated and thickened bronchi and areas of scarring (n = 8) in combination with air trapping and a mosaic pattern (n = 5). In total, 176 spirometries were performed a median of 7 (2-14) times in these 25 patients. In 305 patients who, after screening, were regarded as not having BO, 1001 spirometries (median 3.5 [2-11] per patient) were performed. The FEV_1 value at the time of BO diagnosis was median 49% (23%-74%), which was a decline from baseline (median 49% [24%-81%]). The FEV_1/FVC ratio was median 61% (35%-69%). The median values of FEF_{50} and FEF_{75} were 25% (6%-43%) and 18% (6%-48%) of predicted, respectively. In 6 patients, FEF_{50} was reduced, and in 7 patients, FEF_{75} was reduced already in the spirometry preceding the 1 that—according to the NIH criteria—established the diagnosis of BO. In these patients, at least 2 (range: 2-12) spirometries were performed before the diagnostic spirometry. The decline between the preceding spirometry and the 1 before was median 36% (23%-55%) for FEF_{50} and median 29% (14%-81%) for FEF_{75}. The median value for RV was 177% (92%-363%) of the reference values. In 2 patients, RV was <120%. In 1 of these patients, HRCT findings were consistent with BO, and in the other patient, a postmortem biopsy confirmed the diagnosis of BO. TLC was median 97% (79%-138%), and DLC was median 59% (35%-86%) in the 25 BO patients. The reduction in FEV_1, FVC, and FEF values was persistent in all patients. The disease progression was variable, however; for some patients, the majority with early BO, patients had a rapid decline in lung function, whereas others, mostly patients with late-onset BO, had a more protracted course (Figure 1A and 1B).

Risk Factors and Incidences of BO

In the univariate analysis, 5 factors showed an association with BO at P values of ≤.10, and they were introduced into the stepwise-elimination multivariate analysis. These factors were hematologic malignancy (hazard ratio [HR] 5.42, $P = .10$), myeloablative conditioning (MAC) (3.46, $P = .09$), cGVHD (9.24, $P < .0001$), no DLI treatment (4.18, $P = .05$), and receipt of G-CSF after HSCT (2.51, $P = .065$). Only cGVHD (HR 9.83, 95% confidence interval [CI] 3.42-28.2, $P < .0001$) and DLI (0.18, 0.04-0.77, $P = .02$) were found to be significantly associated with BO in the multivariate analysis. When cGVHD and DLI were treated as time-dependent variables and were introduced into Cox proportional hazards model, both factors were still found to be statistically significant (cGVHD 2.50, 1.55-4.02, $P < .001$) and (DLI 0.50, 0.26-0.94, $P = .03$). The cumulative incidences of BO in patients with DLI treatment (n = 126) or without DLI treatment (n = 401) were 1.6% and 6.6%, respectively, and they were 11.4% and 1.1% in patients with and without cGVHD. The incidences of BO in patients with and without these risk factors are shown in Figure 2. Only 2 patients developed BO after DLI treatment; both received only 1 dose of DLI.

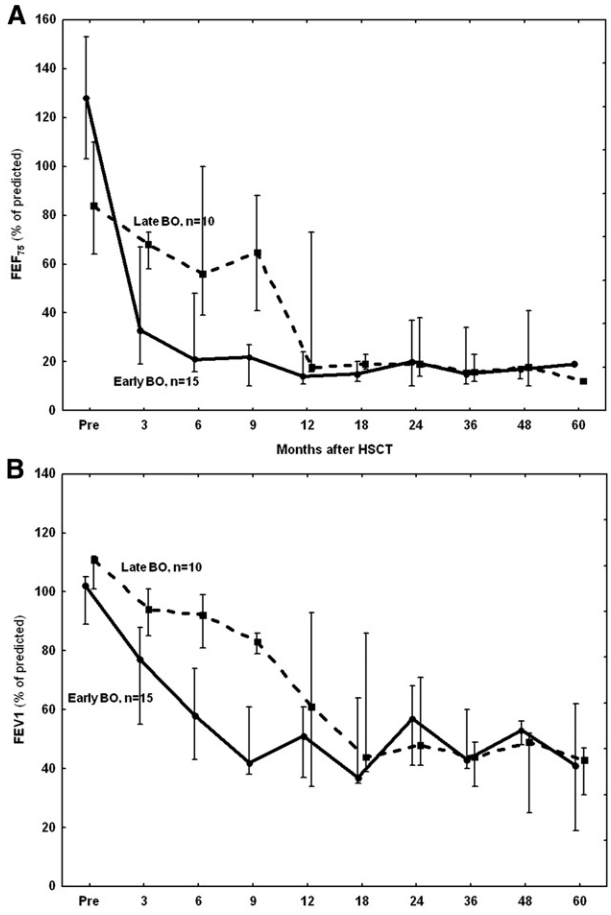

Figure 1. Forced expiratory flow at 75% (FEF_{75}) of forced vital capacity (FVC) (A) and forced expiratory volume in 1 second (FEV_1) (B) in patients with early-onset bronchiolitis obliterans (BO) (within 1 year of allogeneic HSCT) and late-onset BO. Median and 25% to 75% quartile range.

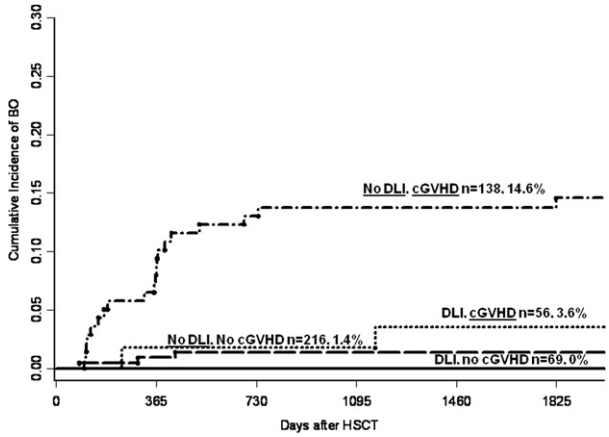

Figure 2. Cumulative incidences of bronchiolitis obliterans (BO) with and without the risk factors chronic graft-versus-host disease (cGVHD) and treatment with donor lymphocyte infusion (DLI).

Patients with Early and Late BO

As immunosuppressive treatment after HSCT, CsA and MTX were administered to all of the 25 patients with BO. Before the diagnosis of BO, CsA had been administered a median of 5.5 (2-12) months to 15 patients with early BO, and a median of 13.5 (4-60) months to 10 patients with late BO. Because of GVHD, steroids had been administered to patients with early BO a median of 5.5 (0-12) months before the diagnosis of BO, and after establishment of the diagnosis, they were continued, increased, or initiated in all of these patients. For a 5-year follow-up time after the diagnosis, CsA was given for a median of 8 (0-60) months to 13 of 15 patients with early BO, and for a median of 43 (0-60) months to 8 of 10 patients with late BO. Likewise, steroids were administered for a median of 12 (0.5-49) months to those with early BO, but only to 4 of the 10 patients with late BO for a median of 54.5 (22-60) months. Other therapies used for patients with early and late BO were, respectively, cimetidine (n = 14 and n = 8), omeprazole (n = 1 and n = 2), anticholinergics, bronchodilators, and/or inhaled steroids (n = 13 and n = 9), psoralen and ultraviolet light A therapy (PUVA) (n = 6 and n = 6), mycophenolate mofetil (MMF) (n = 3 and n = 1) for 5, 12, 20, and 24 months, tacrolimus and sirolimus (n = 1 and n = 3) for 3, 24, 36, and 37 months, or the IL-2 antagonist daclizumab (n = 1 and n = 3). In patients with early BO, FEV_1 was median 57% (37%-74%) and FEV1/FVC was 62% (35%-82%) at the time of diagnosis of BO. In the spirometry preceding the NIH diagnostic spirometry, FEV_1 was 95% (59%-112%) and FEV_1/FVC was 83% (65%-100%). In patients with late BO, FEV_1 was median 42% (23%-60%) and FEV1/FVC was median 59% (36%-67%) at the time of diagnosis of BO, whereas in the preceding spirometry, FEV_1 was median 89% (64%-102%) and FEV_1/FVC was median 78% (66%-93%). The NIH criteria for BO were not fulfilled in any of the spirometries preceding the NIH diagnostic spirometry.

Outcome in BO Patients

Eleven patients with BO (44%) died, 7 of them because of BO in combination with cGVHD and/or infection. The overall 5-year survival in patients with BO (n = 25) was 64%, compared to 50% in those without BO (n = 502). Survival 5 years after diagnosis of BO was 38% and 80% in patients with early BO (within 1 year of HSCT, n = 15) and late BO (n = 10) BO, respectively ($P = .06$) (Figure 3). In patients with or without DLI treatment, survival at 5 years was 35% and 55% ($P = .005$). The 5-year survival for patients treated with DLI because of chimerism, relapse, and graft failure was 65%, 20%, and 17%, respectively. Median survival after DLI treatment was 416 (2-4280) days.

DISCUSSION

The reported incidence of BO is inconsistent, because uniform diagnostic criteria have yet to be established; the reported incidence range is 0% to 48%

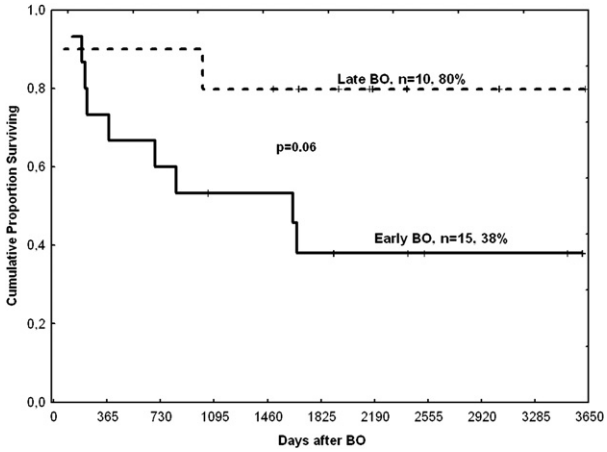

Figure 3. Overall survival (OS) after diagnosis of bronchiolitis obliterans (BO) in patients with early-onset BO (within 1 year of HSCT) and late-onset BO.

[24], but in our study, the incidence was 4.8%. The reason for the wide differences in incidence may be that the term BO has been applied to different histopathologic patterns of bronchiolar fibrosis as well as various clinical syndromes ranging from an irreversible disease with progressive air flow obstruction (BO) to bronchiolitis obliterans organizing pneumonia (BOOP), an infiltrative, acute, and reversible process associated with restricted lung volume [10,25]. Although BO may be a noninfectious disorder, it still complicates a variety of pulmonary infections [26,27]. Viral infections, particularly adenovirus and parainfluenzavirus, have been frequently implicated [26-28]. No viral infections preceded the BO diagnosis in this study, but 4 patients had had culture-verified bacterial infections that may have initiated the process.

According to the NIH consensus document of criteria for cGVHD, 1 of the criteria for BO is evidence of air trapping or small airway thickening, bronchiectasies on HRCT, RV >120%, or pathologic confirmation of constrictive bronchiolitis [12]. In our study, repeated HRCTs were performed in 22 of the 25 BO patients. Only 8 patients (32%) had radiologic changes in HRCTs, associated with BO. Despite the poor diagnostic value of HRCTs in our study, the NIH criteria were met, because in 23 of 25 cases, RV was >120%, and in 2 other patients, HRCT and pathological findings confirmed the diagnosis. The NIH consensus document also states that the only diagnostic manifestation of cGVHD is biopsy-proven BO [12]. However, adequate lung biopsies are difficult to achieve before death and may result in severe complications in up to 13% of patients with cGVHD and BO [18,22,23]. Instead, in our study, persistent dyspnea and dry cough in the absence of infection at the very time of diagnosis of BO, and a new onset of reduced lung function monitored by spirometries, established the diagnosis. In the literature, and according to the NIH criteria, the most relevant spirometric criteria of BO following allogeneic HSCT, as well as after lung transplantation, appears to be a >20% reduction in FEV_1 from pretransplantation or baseline values, or FEV_1 <75% of the predicted value with FEV_1/FVC <0.7 [7,9,11,29]. In this study, in which lung function tests from 330 patients were thoroughly evaluated, the patients diagnosed with BO fulfilled all of these criteria.

In this study, dynamic spirometries and lung volume tests were performed in all BO patients. Lung volume tests can, however, be difficult to perform sometimes, for instance, in children and in patients with an impaired performance status. Thus, we believe that FEV_1 is the most reliable and easy way to detect and monitor the way BO develops, which is in accordance with previous findings [18]. Furthermore, some studies on lung-transplanted patients have suggested that a reduction in FEF of between 25% and 75% of FVC may precede the decline in FEV_1 [30-32]. In this study, 7 patients (28%)—in whom at least 2 (2-12) spirometries were performed before BO was diagnosed, and who were monitored by spirometry every 3 to 6 months for at least 2 years and then annually—had a reduced FEF_{75} even before the NIH spirometry criteria for BO were fulfilled. The FEF values depend on the lung volume and are sensitive (but nonspecific) indicators of subsequent development of BO, and should therefore be interpreted with caution. Even so, regular monitoring of FEF_{50} and FEF_{75} and intraindividual comparisons of the FEF values may serve as an early warning of BO [32].

Even though the incidence of GVHD and air flow obstruction has been reduced after the introduction of CsA as GVHD prophylaxis [33], and lower incidences of BO have been reported after RIC compared to after myeloablative conditioning [34], efforts to treat BO have been disappointing. In the univariate analysis, we found an association between RIC and less BO (relative humidity [RH] 3.46, $P = .09$), but this association did not hold in the multivariate analysis. Some studies have suggested that the use of statins or maintenance macrolide therapy may have a role in the treatment of BO [35-37], as they may halt the disease progression. Furthermore, GVHD-associated esophagitis with recurrent aspirations has also been taken into account as a possible cause of BO [1]. After establishment of the diagnosis of BO, all 25 patients in our study received antacids, and 22 (88%) received oral steroids, anticholinergics, bronchodilators, and/or inhaled steroids. Bronchodilators have been shown to affect clinical improvement and to increase FEV_1 values [3]. In this study, we found that in 13 patients (52%), treatment with (especially) oral or inhaled steroids may have contributed to an increased (n = 4) or at least temporarily stabilized (n = 9) FEV_1. Eleven of these patients are still alive. Treatment with PUVA, tacrolimus, and IL-2 antagonists may also

have been of some benefit, because 5 of the 12 patients who received PUVA (all 4 patients who received tacrolimus and 3 of the 4 who received IL-2 antagonist) are still alive.

It is well known that BO is associated with considerable mortality [7,18,38,39]. However, there is some published evidence that some patients may develop a more slowly progressing or temporarily reversible disease [7,38]. When comparing early and late BO, we found that the mortality was higher in those with early BO (60%) than in those with late BO (20%). The 5-year survival for patients with early-onset BO in our study was nearly the same as in other studies [18,38], but it was high for those with late-onset BO. One explanation of the better outcome in patients with late-onset BO could be their better immune competence after more than a year compared to the first few months after HSCT [40]. Furthermore, patients with late-onset BO had had a more protracted treatment with CsA and steroids at the time of diagnosis than patients with early-onset BO (CsA for a median of 13.5 versus 5.5 months and steroids for a median of 12 versus 5.5 months). However, regardless of whether they had late or early onset, the survivors ended up with equally poor lung function. There was no significant difference in treatment regimens or time from diagnosis of BO to start of steroids and other treatments in these cohorts.

In the multivariate risk factor analyses, cGVHD was significantly associated with development of BO ($P < .001$) and occurred in 88% of the patients with BO, compared to 34% in patients without BO. Chronic GVHD has also been found to be a risk factor for BO in several other studies. However, in most of these studies, cGVHD was combined with other risk factors [6,9,38,39,41-43]. Instead, we found a negative correlation between DLI treatment and BO. DLI has been shown to clear pre-existing infections or to protect from development of new infections [44]. Our study suggests that DLI may also protect against BO even though this is a noninfectious disease. One possible explanation of the unexpected protective role of DLI could be that DLI treatment changes the cytokine profile towards a Th1 profile, which is different from the Th2 profile believed to occur in cGVHD. Still, a protective role for DLI in BO may be controversial and speculative. For example, patients may die early after DLI; however, in this study, only 30 (24%) of the patients given DLI died within 3 months of DLI, and 67 patients (53%) survived more than 1 year after DLI. Further studies with larger patient material are needed to determine the possible influence of a protective role of DLI.

We believe that future studies should concentrate on trying to explore the underlying etiological mechanisms of BO in order to diagnose the condition as soon as possible and to find adequate treatment regimens. One factor that has been suggested to have potential importance for an early diagnosis is Clara cell 16-kDa secretory protein (CC16) [45]. Low or decreasing levels of CC16 (by more than 40% compared to previous samples) have been found to be associated with development of BO [45]. A better understanding of allograft pathogenesis could hopefully lead to an improved ability to predict the likelihood of progression of BO in individual recipients, and thereby help to decide the best possible intervention for each patient. This strategy will hopefully avoid potentially toxic multidrug therapies.

To summarize, BO is a serious, progressive disease associated with a high mortality, especially if it occurs within 1 year of HSCT. The most important diagnostic and monitoring factor was found to be FEV_1, but FEF values may also be of benefit to alert the clinicians to perform a more careful follow-up. Chronic GVHD is a well-known risk factor, but unexpectedly, DLI treatment may have a protective role.

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CONFLICTS OF INTEREST

Dr. Forslöw has no conflict of interest. Dr. Remberger has no conflict of interest. Dr. Gustafsson has no conflict of interest. Dr. Mattsson has no conflict of interest.

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